Alberto de Elzaburu
Alfonso D Rivera Elzaburu
Miguel A Baz
Enrique Armijo
Germán Burgos
Luis H de Larramendi
Doris Bandín
Roberto Martínez
Antonio Tavira
Antonio Castán
Ignacio D Rivera Elzaburu
Jesús Gómez Montero
Pablo González-Bueno

Javier Cervera
Begoña Larrondo
Heinrich Möhring
Juan Antonio Rubiano
José Manuel Cruz
Luis Beneyto
Xavier Lamíquiz
José Ignacio San Martín
Miguel Ángel Medina
Manuel Illescas
Luis Baz
Ramón Cañizares
Víctor Carbayo
Enrique Armijo Ch

Javier Fdez-Lasquetty
Concepción Chacón
Ana Donate
Catherine Bonzom
Juan José Caselles
Fernando Ilardia
Rosa Torrecillas
Laura Alonso
Javier Úbeda-Romero
Pedro Saturio
Luis Soriano
Juan M Sáinz de Marles
Francisco J Sáez
Carlos Morán M
Juan Antonio Romero
Sofía D Rivera Elzaburu

I Arocas
A Vila
L Moraleda
G Armijo
M Glez Gordon
C Sanz
M Vázquez
M García Muñoz
M Amores
R de la Colina
I Navarro

Continuadores de
Julio de Vizcarrondo 1865-1889
F de Elzaburu Vizcarrondo 1880-1921
Alberto de Elzaburu F 1920-1974
Oscar de Elzaburu F 1924-1985
Oficina Vizcarelza Sres Elzaburu

Abogados y Agentes
P. Industrial e Intelectual

Agentes Europeos de Patentes
European Patent Attorneys

Agentes Europeos de Marcas
ante la OAMI/OHIM Alicante
European TM Attorneys

Ingenieros, Biólogos
Físicos y Químicos

Agente Registrador .ES (ESNIC)
Traductores Jurados

Telegramas: VIZCARELZA
Teléfono: (34) 91 700 9400
Telefax: (34) 91 319 3810
Videoconf: (34) 91 702 0786
Correo-e: elzaburu@elzaburu.es
Pág web: www.elzaburu.es

Miguel Ángel, 21
28010 Madrid, España

S/Your ref
DETECCION DE MUTACIONES

N/Our ref
MIT/PCT-154

European Patent Office
D-80298
München

ALEMANIA

Attn. Mr. Mossier, B.
(Authorized Officer)

19 April 2005

Fax no.: 00 49 8923994465

Re: Reply to written opinion
International application no. PCT/ES2004/070001

Dear Sirs,

We reply in due time to the WO by submitting a new set of amended claims wherein all the objections raised have been duly considered.

None of the amendments made into the claims goes beyond the IA as formerly filed, being all supported by the original description. Moreover the total number of claims has been limited from 16 in the IA as formerly filed to 14 as now amended.

Support for the amendments

Amended claim 1 is based in former claim 9
Amended claim 2 is based in former claim 10
Amended claim 3 is based in former claims 9 -10 with regard to former claims 2 and 12
Amended claim 4 is based in former claims 9 -10 with regard to former claims 3 and 12
Amended claim 5 is based in former claim 12
Amended claim 6 is based in former claims 9, 10 and 12
Amended claim 7 is based in former claims 7 and 8

Amended claim 8 is based in former claims 11
Amended claim 9 is based in former claim 13
Amended claim 10 is based in former claim 14
Amended claim 11 is based in former claim 15
Amended claim 12 is based in former claim 16
Amended claim 13 is based in former claim 5
Amended claim 14 is based in former claim 6

Re Item IV

Lack of unity of invention (Rules 13.1 and 13.2 PCT)

Amended set of claims cover a single underlying inventive concept: the detection of mutations in the LDL-gene with the aim to use them in diagnosis (in vitro) of FH. In order to detect such a mutations, oligonucleotides hybridazing with them have been developed and thus they form part of the unitary inventive concept too.

Concerning a possible lack of unity "a posteriori", in our opinion, with the set of amended claims it would not be the case. D1 and D2 do not disclose any of the mutations constituting the characterizing part of amended independent claims 1 and 9. Neither any oligonucleotide claimed in independent claim 12 is disclosed in D1 or D2, nor, obviously, any use of such a oligonucleotides, as claimed in independent claim 7, as amended. The "a posteriori" lack of unity objection has therefore, no legal basis in view of the fact that the set of amended claims provided herein, fulfils novelty and inventive step (see below) requirements.

Re Item V

V.2 Inventive Step (Article 33(1) and (3) PCT)

The technical problem solved by the IA is the provision of a new and inventive in vitro assay method and kit for diagnosis of FH based in the detection of new mutations in the LDL-r gene. For that purpose, new oligonucleotides have been developed for hybridizing and thus detecting those new mutations. As other LDL-r gene mutations were already known in the state of the art (D1 and D2), the new assay method and kit must be considered as an alternative to any prior existing method or kit, if any.

Although in other genes, the discovery of new mutations and its relationship with a specific sickness might be a matter of lab routine, this is not the case for the mutations detected in LDL-r gene and the development of FH. As it is mentioned in D2 (Abstract) and in the IA itself (pg. 2 lines 19-26), the relevance of different mutations in said gene depends greatly on each population considered. D2, by way of example, discloses mutations relevant

specifically for FH genetic testing limited to the Netherlands. In the IA all the patient cases studied were Spanish and, probably, in D1 (discussed in the IA, pg. 7, line 32 to pg.8, line 1) all the family history cases genetically tested for mutations, pertain to the Japanese population. Those differences between populations justify by themselves, in our view, the necessity of investigating and developing alternative diagnosis methods and kits. Moreover, prior art disclosed mutations, due to the differences among different genetic populations, hardly may render obvious or suggest mutations relevant or valid for testing FH for nationals other than their own genetic population. That was the case of the mutation disclosed in D1 (pg. 43, lines 7 and 8) , mentioned in the WO. That mutation, detailed in Fig. 7 of D1 (see pg. 5 lines 36 and 37) corresponds to a substitution T>A around nucleotide 283 (it is hard to know the exact position). In amended claim 1, there is not any mutation even close to that position of LDL-r gene sequence.

Neither a combination of D1 and D2, could lead to the man skilled in the art to arrive to the technical solution found in present IA, simply because, as previously mentioned, assays used for genetic testing of FH based on specific mutations for each genetic population, can not serve for genetic testing of FH in other populations, in which other different mutations prevail.

We, therefore, conclude that amended independent claims 1, 7, 9 and 13 are novel and inventive over cited prior art D1 and/or D2 and, subsequently, dependent claims 2 to 6 (depending on claim 1), 8 (depending on claim 7), 10 to 12 (depending on claim 9) and 14 (depending on claim 13) are novel and inventive too.

Clarity and sufficiency of disclosure (Articles 6 and 5 PCT)

1) The term "capable of hybridizing", present in former claims 4 and 5 and objected by the examiner, has been replaced by "hybridizing" in amended claims 1, 3 and 4. Hybridization conditions are common knowledge but, in any case, a detailed description of the hybridization steps is disclosed in the description of the IA (pg. 14, line 6 to pg. 16 line 5 and Fig. 3, legend in pg. 61, lines 10-14).

2) The correction of the mistake made in the transcription of the mutation 2389+3 A>C, in pg. 57 lines 14, 22 and 27 is respectfully requested under Rule 91.1 PCT in due time, in view that the error committed was obvious. A corrected pg. 57 for replacement is attached hereto, wherein wrong transcriptions "C>T" have been duly replaced by the right base substitution "A>C".

This representative, respectfully request under Rule 66.4 PCT to have an additional opportunity to submit further amendments if that IPEA would consider necessary to issue a 2nd WO for any outstanding or new objection to be answered to. Moreover, this representative shall be willing to maintain with the examiner in charge of the IPE for this

IA, as many informal communications, under Rule 66.6 PCT as she/he may consider appropriate, in order to further work out and outstanding doubt or question the present answer may still raise or leave non replied.

Very truly yours,

ELZABURU

Dr. Manuel Illescas

- Amended set of claims 1-14 (Replacement pages 63-68)
- Replacement corrected page 57

AMENDED CLAIMS

1.- Assay kit characterized by comprising oligonucleotides hybridizing with any of the mutations in the gene sequence of LDL-r gene selected from: (-23)A>C, 1054 del11, 108delC, 1197de19, 1207de1T, 1432delG, 191-2delAinsCT, 2184delG, 231delC, 2399del5ins4, 313+linsT, 338dell6, 509insC, 675dell5, 684dup12, 941-39>T, C195R, C255G, C319Y, D157G, D630N, E291X, H635N, N59K, T41M, W515X, Y379X, Y421X, T433N, 818de18, 1423delGC/insA, 1204insT, 451de13, G516X, 2389+4A>G, 1815del11, 1186+5G>A, T740M, I771T, R279G, T446I, H562Q, C74Y, D686Y, G(-2)R, E579D, S205C, D200V, V766E, L(-6)P, 2544insC, C42Y, 2389+3A>C, [1587-5de15;1587del31].

2.- Assay kit according to claim 1 characterized by comprising at least an oligonucleotide selected from: SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:24, SEQ ID NO:29, or at least one from SEQ ID NO:37 to SEQ ID NO:147 or from SEQ ID NO:154 to SEQ ID NO:259.

3.- Assay kit according to any of the claims 1 or 2 characterized by further comprising oligonucleotides hybridizing with any of the mutations in the gene sequence of LDL-r gene selected from: 2393del9, (-42)C>G, (-49)C>T, 1045delC, 1061-8 T>C, A378T, C358R, 1358+1G>A, 1706-10G>A, 1845+1G>C, 2085del19, 2lldelG, 2140+5G>A, 2207insT, 2390-1G>C, 313+1G>C, 313+1G>A, 518delG, 7delC, 872delC, 884delT, 920ins4, A519T, C113W, C255X, C281Y, C297F, C347Y, C371X, C646Y, C677Y, C68W, C74G, C95R, D151N, D200G, D200Y, D280G, E10X, E246A, E256K, F634L, G322S, G352D, G571E, N543H, N804K, Q12X, Q133X, Q357P, Q427X, Q71E, R395Q, R574W, R612C, S156L, S205P, T413K, T7051, V502M, W(-18)X, W541X, D679E, 1359-1G>A, C127R, 681ins21, C122X, V408M, G528D, D412H, N619N, E80K, L534P, L621S, C356Y, R329X, G248D, C201Y,

313+5G>A, C358Y, C331R, D157N, V776M, P664L, W462X, Q328X, L584P, R395W, G314V, W469X, P678L, R612H, R236W.

4.- Assay kit according to any of the claims 1 to 3 characterized by further comprising oligonucleotides hybridizing with any of the polymorphims in the gene sequence of LDL-r gene selected from: 81T>C BstUI Exon 2, 1060+10G>C SmaI Exon 7, 1171G>A StuI Exon 8, 1413G>A DdeI Exon 10, 1617C>T BstNI Exon 11, 1725C>T SSCP Exon 12, 1771C>T HincII Exon 12, 1959 T>C AvaII Exon 13, 2232G>A MspI Exon 15.

5.- Assay kit according to any of the claims 1 to 4 characterized by comprising at least an oligonucleotide selected from: SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:153.

6.- Assay kit according to any of the claims 1 to 5 characterized by having the oligonucleotides coupled to a support.

7.- Use in extracorporeal methods of in vitro detection of LDL-r gene mutations for diagnosis of familial hypercholesterolemia of any of the oligonucleotides selected from: SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:24, SEQ ID NO:29, or at least one from SEQ ID NO:37 to SEQ ID NO:147 or from SEQ ID NO:154 to SEQ ID NO:259.

8.- Use in extracorporeal methods of in vitro detection of LDL-r gene mutations for diagnosis of familial hypercholesterolemia, according to claim 7 of any of the oligonucleotides selected from: SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:24, SEQ ID NO:29, or at least one from SEQ ID NO:37 to SEQ ID NO:147 or from SEQ ID NO:154 to SEQ ID NO:259, in combination with any of the oligonucleotides selected from: SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:153.

9.- Extracorporeal method of in vitro diagnosis of familial hypercholesterolemia (FH) characterized in that in a biological sample of an individual is detected at least one mutation in LDL-r gene, selected from: (-23)A>C, 1054 del11, 108delC, 1197de19, 1207de1T, 1432delG, 191-2delAinsCT, 2184delG, 231delC, 2399del5ins4, 313+linsT, 338dell6, 509insC, 675dell5, 684dup12, 941-39>T, C195R, C255G, C319Y, D157G, D630N, E291X, H635N, N59K, T41M, W515X, Y379X, Y421X, T433N, 818de18, 1423delGC/insA, 1204insT, 451de13, G516X, 2389+4A>G, 1815del11, 1186+5G>A, T740M, I771T, R279G, T446I, H562Q, C74Y, D686Y, G(-2)R, E579D, S205C, D200V, V766E, L(-6)P, 2544insC, C42Y, 2389+3A>C, [1587-5de15;1587del31].

10.- Extracorporeal method of in vitro diagnosis of familial hypercholesterolemia (FH), according to claim 9, characterized in that in a biological sample of an individual, in combination with at least one of the mutations in LDL-r gene selected from: (-23)A>C, 1054 del11, 108delC, 1197de19, 1207de1T, 1432delG, 191-2delAinsCT, 2184delG, 231delC, 2399del5ins4, 313+linsT, 338dell6, 509insC, 675dell5, 684dup12, 941-39>T, C195R, C255G, C319Y, D157G,

D630N, E291X, H635N, N59K, T41M, W515X, Y379X, Y421X, T433N, 818de18, 1423delGC/insA, 1204insT, 451de13, G516X, 2389+4A>G, 1815del11, 1186+5G>A, T740M, I771T, R279G, T446I, H562Q, C74Y, D686Y, G(-2)R, E579D, S205C, D200V, V766E, L(-6)P, 2544insC, C42Y, 2389+3A>C, [1587-5de15;1587del31] is further detected at least one mutation, in the same LDL-r gene, selected from: 2393del9, (-42)C>G, (-49)C>T, 1045delC, 1061-8 T>C, A378T, C358R, 1358+1G>A, 1706-10G>A, 1845+1G>C, 2085del19, 2lldelG, 2140+5G>A, 2207insT, 2390-1G>C, 313+1G>C, 313+1G>A, 518delG, 7delC, 872delC, 884delT, 920ins4, A519T, C113W, C255X, C281Y, C297F, C347Y, C371X, C646Y, C677Y, C68W, C74G, C95R, D151N, D200G, D200Y, D280G, E10X, E246A, E256K, F634L, G322S, G352D, G571E, N543H, N804K, Q12X, Q133X, Q357P, Q427X, Q71E, R395Q, R574W, R612C, S156L, S205P, T413K, T7051, V502M, W(-18)X, W541X, D679E, 1359-1G>A, C127R, 681ins21, C122X, V408M, G528D, D412H, N619N, E80K, L534P, L621S, C356Y, R329X, G248D, C201Y, 313+5G>A, C358Y, C331R, D157N, V776M, P664L, W462X, Q328X, L584P, R395W, G314V, W469X, P678L, R612H, R236W.

11.- Extracorporeal method of in vitro diagnosis of familial hypercholesterolemia (FH), according to any of the claims 9 or 10, characterized in that in a biological sample of an individual, in combination with at least one of the mutations in LDL-r gene selected from: (-23)A>C, 1054 del11, 108delC, 1197de19, 1207de1T, 1432delG, 191-2delAinsCT, 2184delG, 231delC, 2399del5ins4, 313+linsT, 338dell6, 509insC, 675dell5, 684dup12, 941-39>T, C195R, C255G, C319Y, D157G, D630N, E291X, H635N, N59K, T41M, W515X, Y379X, Y421X, T433N, 818de18, 1423delGC/insA, 1204insT, 451de13, G516X, 2389+4A>G, 1815del11, 1186+5G>A, T740M, I771T, R279G, T446I, H562Q, C74Y, D686Y, G(-2)R, E579D, S205C, D200V, V766E, L(-6)P, 2544insC, C42Y, 2389+3A>C, [1587-5de15;1587del31], 2393del9, (-42)C>G, (-49)C>T, 1045delC, 1061-8 T>C, A378T, C358R, 1358+1G>A, 1706-

10G>A, 1845+1G>C, 2085del19, 2lldelG, 2140+5G>A, 2207insT, 2390-1G>C, 313+1G>C, 313+1G>A, 518delG, 7delC, 872delC, 884delT, 920ins4, A519T, C113W, C255X, C281Y, C297F, C347Y, C371X, C646Y, C677Y, C68W, C74G, C95R, D151N, D200G, D200Y, D280G, E10X, E246A, E256K, F634L, G322S, G352D, G571E, N543H, N804K, Q12X, Q133X, Q357P, Q427X, Q71E, R395Q, R574W, R612C, S156L, S205P, T413K, T7051, V502M, W(-18)X, W541X, D679E, 1359-1G>A, C127R, 681ins21, C122X, V408M, G528D, D412H, N619N, E80K, L534P, L621S, C356Y, R329X, G248D, C201Y, 313+5G>A, C358Y, C331R, D157N, V776M, P664L, W462X, Q328X, L584P, R395W, G314V, W469X, P678L, R612H, R236W, is further detected at least one LDL-r gene polymorphism selected from: 81T>C BstUI Exon 2, 1060+10G>C SmaI Exon 7, 1171G>A StuI Exon 8, 1413G>A DdeI Exon 10, 1617C>T BstNI Exon 11, 1725C>T SSCP Exon 12, 1771C>T HincII Exon 12, 1959 T>C AvaII Exon 13, 2232G>A MspI Exon 15.

12.- Extracorporeal method of in vitro diagnosis according to any of the claims 9 to 11, comprising amplifying DNA fragments that contain any mutation selected from: (-23)A>C, 1054 del11, 108delC, 1197de19, 1207de1T, 1432delG, 191-2delAinsCT, 2184delG, 231delC, 2399del5ins4, 313+linsT, 338dell6, 509insC, 675dell5, 684dup12, 941-39>T, C195R, C255G, C319Y, D157G, D630N, E291X, H635N, N59K, T41M, W515X, Y379X, Y421X, T433N, 818de18, 1423delGC/insA, 1204insT, 451de13, G516X, 2389+4A>G, 1815del11, 1186+5G>A, T740M, I771T, R279G, T446I, H562Q, C74Y, D686Y, G(-2)R, E579D, S205C, D200V, V766E, L(-6)P, 2544insC, C42Y, 2389+3A>C, [1587-5de15;1587del31], alone or in combination with any mutation selected from: 2393del9, (-42)C>G, (-49)C>T, 1045delC, 1061-8 T>C, A378T, C358R, 1358+1G>A, 1706-10G>A, 1845+1G>C, 2085del19, 2lldelG, 2140+5G>A, 2207insT, 2390-1G>C, 313+1G>C, 313+1G>A, 518delG, 7delC, 872delC, 884delT, 920ins4, A519T, C113W, C255X, C281Y, C297F, C347Y, C371X, C646Y, C677Y, C68W, C74G, C95R, D151N, D200G, D200Y, D280G, E10X,

E246A, E256K, F634L, G322S, G352D, G571E, N543H, N804K, Q12X, Q133X, Q357P, Q427X, Q71E, R395Q, R574W, R612C, S156L, S205P, T413K, T7051, V502M, W(-18)X, W541X, D679E, 1359-1G>A, C127R, 681ins21, C122X, V408M, G528D, D412H, N619N, E80K, L534P, L621S, C356Y, R329X, G248D, C201Y, 313+5G>A, C358Y, C331R, D157N, V776M, P664L, W462X, Q328X, L584P, R395W, G314V, W469X, P678L, R612H, R236W and/or with any polymorphism selected from: 81T>C BstUI Exon 2, 1060+10G>C SmaI Exon 7, 1171G>A StuI Exon 8, 1413G>A DdeI Exon 10, 1617C>T BstNI Exon 11, 1725C>T SSCP Exon 12, 1771C>T HincII Exon 12, 1959 T>C AvaII Exon 13, 2232G>A MspI Exon 15, by the technique of the chain reaction of the polymerase (PCR), by using any of the oligonucleotides selected between SEQ ID NO:2 to SEQ ID NO:259 or combinations thereof, subjecting the PCR products to an analysis by the simple chain conformation polymorphisms technique (SSCP), sequencing those fragments having an anomalous pattern by SSCP to detect the mutations that would be identified subsequently by restriction analysis or by means of any of the assay kits of claims 1 to 6.

13.- Oligonucleotides hybridising with any of the mutations selected from: (-23)A>C, 1054 del11, 108delC, 1197de19, 1207de1T, 1432delG, 191-2delAinsCT, 2184delG, 231delC, 2399del5ins4, 313+linsT, 338dell6, 509insC, 675dell5, 684dup12, 941-39>T, C195R, C255G, C319Y, D157G, D630N, E291X, H635N, N59K, T41M, W515X, Y379X, Y421X, T433N, 818de18, 1423delGC/insA, 1204insT, 451de13, G516X, 2389+4A>G, 1815del11, 1186+5G>A, T740M, I771T, R279G, T446I, H562Q, C74Y, D686Y, G(-2)R, E579D, S205C, D200V, V766E, L(-6)P, 2544insC, C42Y, 2389+3A>C, [1587-5de15;1587del31].

14.- Oligonucleotides according to claim 13 selected from: SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:24, SEQ ID NO:29, or at least one from SEQ ID NO:37 to SEQ ID NO:147 or from SEQ ID NO:154 to SEQ ID NO:259.